

THG113: A Novel Selective FP Antagonist that Delays Preterm Labor

Krishna G. Peri,† Christiane Quiniou,* Xin Hou,* Daniel Abran,†
Daya R. Varma,‡ William D. Lubell,§ and Sylvain Chemtob*‡

PGF_{2α} is an important smooth muscle contractile agent that exerts significant effects on myometrium and is implicated in labor. THG113 was recently identified as a PGF_{2α} receptor (FP) antagonist. We characterized the specificity and selectivity of THG113, tested its effects on PGF_{2α}-induced smooth muscle contraction, and assessed its efficacy in a model of endotoxin (LPS)-induced preterm labor. [¹²⁵I]THG113 bound specifically to FP-expressing but not to native (not expressing FP) HEK293 cells. In FP-expressing HEK293 cells, THG113 markedly reduced PGF_{2α}-elicited phosphoinositide hydrolysis (IC₅₀ 27 nM). Similarly, PGF_{2α}-evoked microvascular (retinal) contraction was noncompetitively blocked (by >90%) by THG113. In contrast, contraction to agonists of homologous prostanoid receptors EP₁ and TP (17-phenyl-trinor PGE₂ and U46619) was unaffected (<1%) by high concentrations of THG113 (100 μmol/L); THG113 (100 μmol/L) also did not affect contraction to numerous other agents including platelet activating factor, endothelin, and angiotensin II. Force and duration of PGF_{2α}-evoked contractions of myometrial strips of pig (non-pregnant, luteal phase) and mouse (immediately postpartum) were markedly reduced by THG113. In an endotoxin-induced preterm mouse model, lipopolysaccharide (50 μg intraperitoneal) injection at 16 days' gestation resulted in 100% delivery within 15 h; in contrast, 70% of those treated with THG113 (1 mg/day) delivered >24 h later (at 18 days' gestation; term: 19 days). In addition, in mice injected with lipopolysaccharide and treated 6 h later with THG113 (0.1 mg bolus followed by 1 mg/day) 40% delivered >48 h later. Fetuses of pregnant mice treated with THG113 were born alive, had higher birth weights (1.6 ± 0.1 v 1.4 ± 0.05 g), and appeared healthy. This study describes an effective and selective noncompetitive FP antagonist, THG113, which significantly delays preterm delivery; this provides the basis for future investigations for its use in tocolysis.

Copyright 2002, Elsevier Science (USA). All rights reserved.

Actions of specific prostaglandin synthases results in the formation of 5 major prostanoids, PGE₂, PGF_{2α}, PGD₂, PGI₂, and TXA₂. They exert their effects by acting on specific G protein-coupled receptors; PGE₂ via EP₁₋₄, PGF_{2α} via FP, PGD₂ via DP, PGI₂ via IP and TXA₂ via TP.¹ Diverse effects of PGs in mammalian physiology are well described, and different prostaglandins produce opposing effects on smooth muscle contraction; for instance, PGI₂ and PGD₂ are relaxants, whereas PGF_{2α} and TXA₂ are constrictors; PGE₂ has different effects depending on the receptor distribution and composition in the tissue. In many tissues and conditions, more than 1 type of prostaglandin is formed. At present, inhibiting cyclooxygenase activity by nonsteroidal anti-inflammatory drugs (NSAIDs) is the only available means of therapy in conditions where blockade of prostanoid action is desired. But, nonselective inhibition of prostanoid action may produce undesired ac-

tions. Thus prostanoid receptor-selective drugs offer a more effective therapy in variety of medical conditions.

PGF_{2α} is the most effective smooth muscle contractile prostaglandin.¹ PGF_{2α} stimulates myometrial contraction in several species^{2,3} as

From the *Departments of Pediatrics, Ophthalmology and Pharmacology, Research Center of Hôpital Ste. Justine, Montréal, Québec, †Theratechnologies Inc, St. Laurent, Québec, ‡Department of Pharmacology & Therapeutics, McGill University, Montréal, Québec, §Department of Chemistry, Université de Montréal, Montréal, Québec.

S.C. is a recipient of a Canadian Institutes of Health Research Scientist Award and is an awardee of a Canada Research Chair (perinatology).

Address reprint requests to Sylvain Chemtob, MD, PhD, FRCPC, Departments of Pediatrics, Ophthalmology and Pharmacology, Research Center, Hôpital Ste. Justine, 3175 Côte Ste. Catherine, Montréal, Québec H3T-1C5; e-mail: sylvain.chemtob@umontreal.ca.

Copyright 2002, Elsevier Science (USA). All rights reserved.

0146-0005/02/2606-0002\$35.00/0

doi:10.1053/sjpr.2002.37307

other prostaglandins, namely PGE_2 , may cause relaxation in lower segments of the uterus.^{2,4} Indeed, $\text{PGF}_{2\alpha}$ plays a pivotal role in parturition as indicated by several lines of evidence: 1) A significant rise in $\text{PGF}_{2\alpha}$ levels in amniotic fluid during early labor^{5,6}; 2) $\text{PGF}_{2\alpha}$ stimulates uterine contractility² and induces labor⁷; 3) $\text{PGF}_{2\alpha}$ receptor expression augments markedly during term and especially preterm parturition as part of the contraction-associated proteins (which include actin, myosin, connexin-43, receptors for oxytocin and prostaglandins, and possibly COX-2)^{4,8-11}; 4) Effects of the major uterotonic oxytocin are partly mediated by $\text{PGF}_{2\alpha}$ and the latter in turn augments oxytocin receptor expression^{12,13} such that oxytocin and prostaglandins are closely intertwined³; 5) Inhibition of prostaglandin formation reduces myometrial contractions and prolongs labor^{14,16}; and 6) Disruption of the $\text{PGF}_{2\alpha}$ receptor gene leads to failure of animals to go into labor.¹⁷

Although numerous $\text{PGF}_{2\alpha}$ receptor agonists have been developed,^{18,19} until now there have been no selective antagonists. THG113 is a rationally designed compound and was recently reported to exhibit selective FP receptor antagonism.²⁰ Therefore, we planned to characterize the specificity and selectivity of THG113 on FP-expressing cells and on smooth muscle and determine its efficacy in a model of endotoxin-induced preterm labor.

Materials and Methods

Transfection of FP Receptor cDNA in Human Embryonic Kidney 293 Cells

The full-length coding sequence of human FP was subcloned into the mammalian expression vector pRcCMV (Invitrogen) using methods reported previously.²¹ The plasmid was prepared with a Qiagen plasmid preparation kit and 2 μg of DNA were transfected into human embryonic kidney (HEK) cells with Lipofectamine (Life Technologies, Burlington, Ontario, Canada). The transfectants were selected by using G418 (1 mg/mL) for 2 weeks. Polyclonal population of FP transfectant cell line (FP/293) were grown in Dulbecco's Modification of Eagles Medium complete medium containing G418 (0.2 mg/mL) at 37°C in a humidified atmosphere at 5% CO_2 in air.

THG113 Binding Assay

FP/293 cells were trypsinized and washed with binding buffer (sodium phosphate buffer 0.01 mol/L pH 7.4; NaCl 0.1 mol/L; MgCl_2 0.002 mol/L; acetylsalicylic acid 0.02% p/v; benzamidine 0.001 mol/L; EGTA 0.0005 mol/L; glucose 0.9 mg/mL and protease inhibitor cocktail (Roche Biochemical, Burlington, NC). Binding was performed at 37°C for 30 min with 10^5 cells, [^3H] $\text{PGF}_{2\alpha}$ (20 nmol/L final; specific activity 212 Ci/mmol) or with [^{125}I]THG113 (100,000 cpm final) and 10^{-5} to 10^{-10} mol/L of cold $\text{PGF}_{2\alpha}$ or THG113. The reaction was started by adding the cells for a final reaction volume of 100 μL and stopped by 3 washes of 4 mL of stop buffer (cold Tris-HCl 50 mmol/L pH 7.4). The reaction mix was then filtered through GF/B filters. Radioactivity on filters was counted with a scintillation counter. Specific binding was the difference between the binding in the absence and in the presence of unlabeled ligands.

Phosphoinositide Hydrolysis Assay

FP/293 grown in 12 well plates were labeled with 1-2 $\mu\text{Ci/mL}$ [^3H]-myo inositol (17 Ci/mmol; Amersham Canada) overnight. The cells were preincubated in DMEM containing 10 mmol/L LiCl with different concentrations of THG113 for 30 minutes at 37°C. Cells were then stimulated with 100 nmol/L $\text{PGF}_{2\alpha}$ for 30 min at 37°C and the reaction was terminated by addition of 0.5 volume of NaOH (0.1 N), followed by acidification with 0.2 N formic acid. Cells were collected by scraping and 0.4 mL each of methanol and chloroform were added. Total inositol phosphates were separated by using Dowex AG1X8 (formate form) and 1.2 mol/L ammonium formate in 0.1 N formic acid as the eluant. Radioactivity of phosphoinositides was determined by liquid scintillation method.

Vascular Contraction Preparations

Retinal eyecup preparations for measuring vasomotor responses were prepared as described.^{22,23} FP receptor densities were augmented by treating newborn pigs with ibuprofen (30 mg/kg every 8 h for 24 h).²⁴ Eyes were immediately removed and placed in ice-cold Krebs buffer of the following composition (mmol/L): NaCl, 120; KCl, 4.5; CaCl_2 , 2.5; MgSO_4 , 1.0; NaHCO_3 , 27; KH_2PO_4 , 1.0; sodium

edetate, 0.01; glucose, 10; and heparin (1.5 U/mL). After removal of the anterior segment the eyecup was pinned to a wax support in a 20-mL Krebs buffer bath equilibrated with 95% O₂ and 5% CO₂, and maintained at 37°C and pH 7.35-7.45. Dose-responses of various vasoactive compounds in the presence/absence of THG113 on retinal vasomotricity were recorded by video imaging as described in detail.²²⁻²⁴ The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400, Thornwood, NY) and the responses were quantified by a digital image analyzer (Sigma Scan Software; Jandel Scientific, Corte Madera, CA). Vascular diameter was recorded before and 5 min after the topical application of the agonist.

Myometrial Contraction Preparations

Porcine uterine tissues were collected from animals during luteal phase (by ovarian examination) from a local abattoir (St-Hélène, Québec). Uterus from mice were obtained from animals immediately after term delivery. Myometrial strips (2 to 3-mm wide and 1 to 2-cm long) from both species were suspended in organ baths containing Krebs buffer equilibrated with 21% oxygen at 37°C as described previously.²⁵⁻²⁷ Initial tension was set at 2 g. After 1 h of equilibration, changes in mean basal tension, as well as peak, duration and frequency of spontaneous contractions in response to added agents were recorded with a Kent digital polygraph system.

Endotoxin-induced Preterm Labor Mouse Model

Timed-pregnant CD-1 mice at 16 days' gestation (term: 19.2 days) were anesthetized with ketamine (100 mg/kg intramuscular) and xylazine (5 mg/kg intramuscular). Primed osmotic pumps (Alzet pump, Alzet, Cupertino, CA) containing either saline or THG113 (1 mg/day/animal) were subcutaneously implanted on the backs of the animals; infusion of the peptide was immediately preceded by bolus injection of THG113 (0.1 mg/animal intraperitoneal). Within 15 min after placement of the pumps, the animals were injected with lipopolysaccharide ([LPS] E-coli endotoxin, 50 µg/animal in-

traperitoneal) to mimic the infectious component in human preterm labor.^{28,29} In a separate group of animals LPS was injected 4 to 7 h prior to administration of THG113. Animals were in-

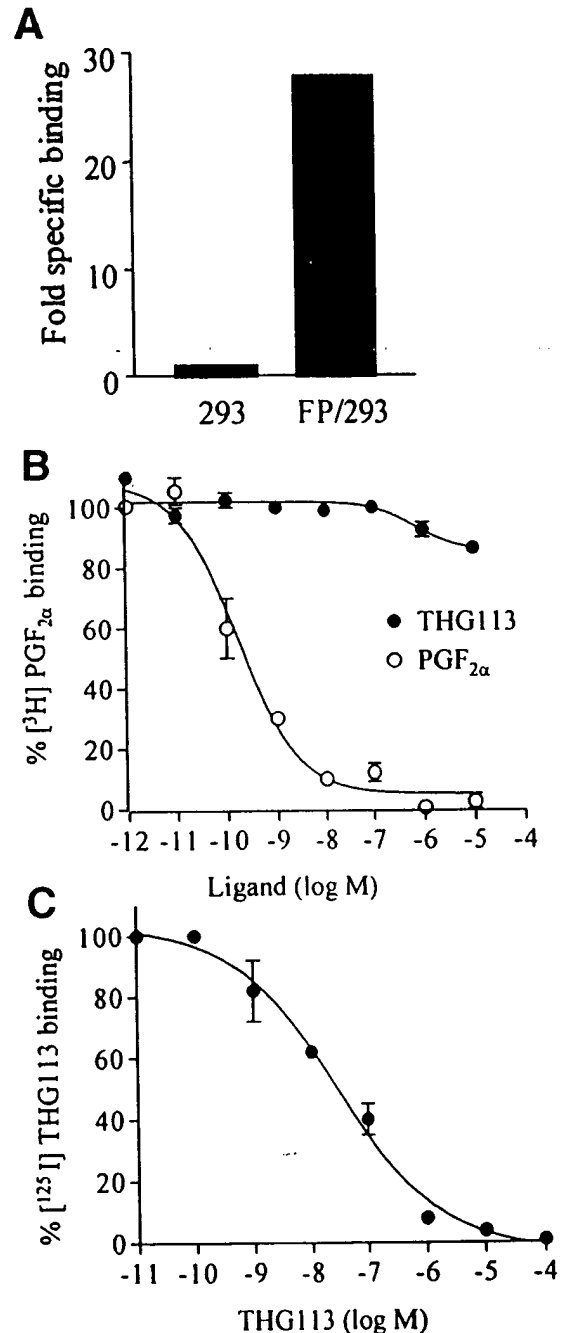


Figure 1. Binding of THG113 to FP/cells. A) Specific binding of [¹²⁵I]THG113 to FP/293 cells. B) Displacement of [³H]PGF_{2α} by PGF_{2α} and THG113. Values are mean ± SEM. C) Displacement of [¹²⁵I]THG113 by THG113.

spected every hour for the first 18 h and every 2 h thereafter to document the timing of birth.

Statistical Analysis

Data were analyzed by one- or two-way ANOVA factoring for treatment group or drug concentration, followed by the Tukey-Kramer method for comparison among means. Nonparametric analysis was performed by Fisher exact test. Statistical significance was set at $P < .05$.

Results and Discussion

Binding of THG113

FP/293 cells exhibited specific binding of [125 I]THG113; [125 I]THG113 did not bind to parent HEK293 cells (Fig 1C). Binding of [125 I]THG113 was fully displaced by THG113 with an $IC_{50} = 35$ nM (Fig 1B). To determine if THG113 bound to the same site as $PGF_{2\alpha}$ on FP/293 cells, displacement of specifically bound [3 H] $PGF_{2\alpha}$ by THG113 was measured in FP/293 cells. [3 H] $PGF_{2\alpha}$ was negligibly displaced by THG113 but fully displaced by $PGF_{2\alpha}$ ($IC_{50} = 0.16$ nmol/L; Fig 1B), suggesting distinct binding sites for THG113 and $PGF_{2\alpha}$ on FP.

Effect of THG113 on Inositol Phosphate Production

FP/293 expressed a functional FP receptor; accordingly, $PGF_{2\alpha}$ augmented phosphoinositide hydrolysis (Fig 2A).^{1,30} THG113 inhibited $PGF_{2\alpha}$ -induced inositol phosphate formation with an $IC_{50} = 27$ nmol/L (Fig 2B); THG113 (100 nmol/L) maximally inhibited inositol phosphate generation (Fig 2C).

Effects of THG113 on Vasomotor Response to $PGF_{2\alpha}$

The efficacy of THG113 was tested in newborn porcine retinal vasculature known to express FP after ibuprofen treatment.²⁴ $PGF_{2\alpha}$ elicited a strong vascular contraction; this effect was nearly

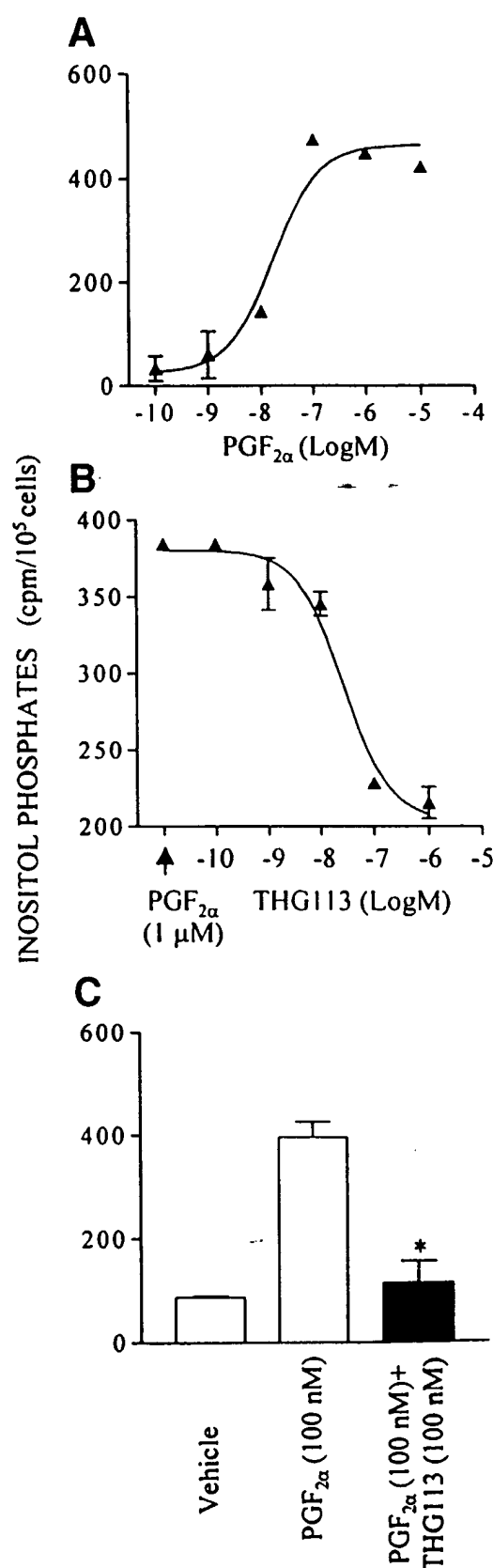


Figure 2. Effect of THG113 on $PGF_{2\alpha}$ -induced inositol phosphate production in FP/293 cells. A) Effect of $PGF_{2\alpha}$ on inositol phosphate production. B) and C) Inhibition of $PGF_{2\alpha}$ -induced inositol phosphate production by THG113. Values are mean \pm SEM. * $P < .05$ compared to corresponding cells untreated with THG113.

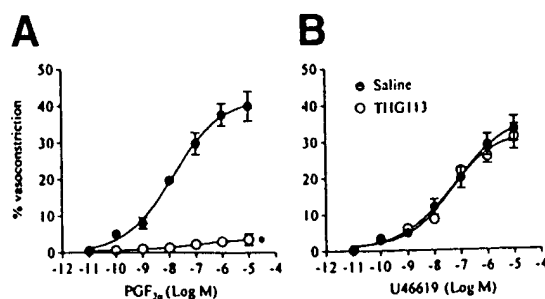


Figure 3. Effect of THG113 on A) PGF_{2α}- and B) U46619-induced retinal vasoconstriction.

completely blocked by 100 μ M THG113 (Fig 3A) even at high concentrations of PGF_{2α}; the vasomotor responses to PGF_{2α} were fully recovered by the removal of THG113 from the buffer (data not shown). Distinct site of binding (Fig 1B) and the inability of the agonist to displace THG113 (Fig 3A) suggested a noncompetitive mode of inhibition of FP receptor by THG113. In contrast, contraction to U46619, the TXA₂ mimetic and an agonist of TP receptor (homologous to FP),³⁰ was unaffected by THG113 (Fig 3B and Table 1).

We further evaluated the selectivity of THG113 to FP by measuring the retinal vasomotor responses of various agents (Table 1). High concentrations of THG113 (100 μ mol/L) did not antagonize effects of numerous constrictors including other lipids, catecholamines, pep-

Table 1. Selective Inhibition of Retinal Vasoconstriction by THG113

Agonist (Target)	Percent Inhibition of Retinal Vasomotor Responses
PGF _{2α} (FP)	90.4
17-phenyl trinor PGE ₂ (EP ₁)	5.0
U46619 (TP)	<1
C-PAF (PAF-R)	4.0
Phenylephrine (α 1 adrenoceptor)	<1
Urotensin II (GPR14)	<1
Endothelin (ET)	<1
Angiotensin II (AT)	<1
BHQ (sarcoplasmic Ca ⁺⁺ ATPase)	<1

NOTE.

Constriction of FP-containing retinal vasculature^{22,24} was studied by video-imaging technique, as described in Materials and Methods. Dose-response to agonists (0.1 μ mol/L) were studied in absence and presence of THG113 (100 μ mol/L).

tides as well as to sarcoplasmic calcium ATPase inhibitor BHQ (Table 1); more importantly, THG113 minimally inhibited constriction to 100 nmol/L 17-phenyl trinor PGE₂, the agonist of EP₁-most homologous prostanoid receptor to FP.³⁰ These data suggested that THG113 is a selective inhibitor of FP receptor.

Inhibition of Myometrial Contractility by THG113

In nonpregnant porcine myometrial tissues, THG113 diminished PGF_{2α} (1 μ mol/L)-in-

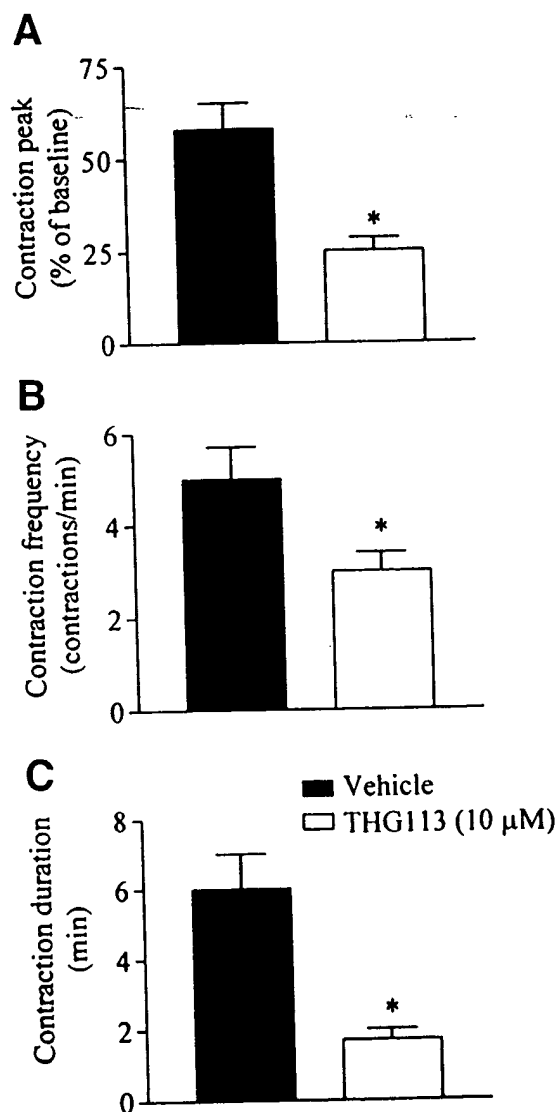


Figure 4. Modulation of PGF_{2α} (1 μ mol/L)-induced porcine myometrial contractions by THG113. Values are mean \pm SEM. * P < .05 compared to vehicle-treated tissues.

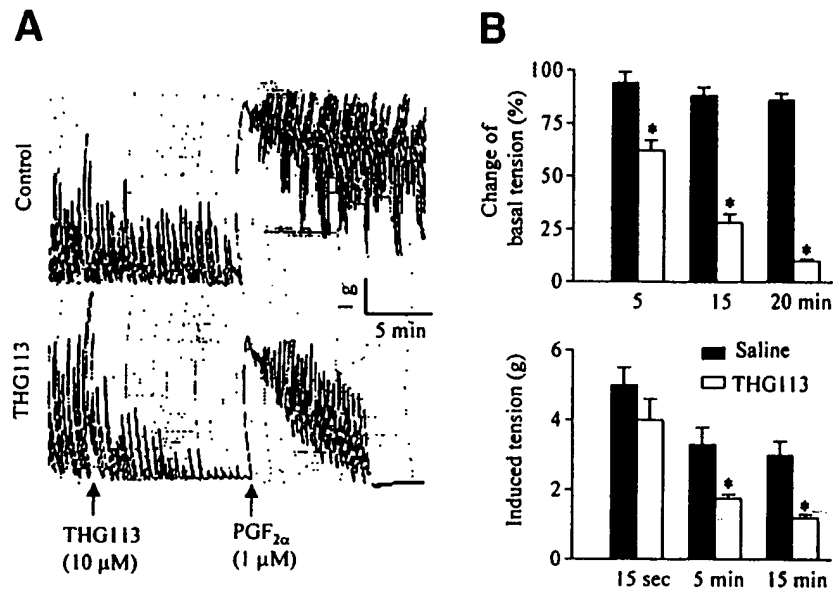


Figure 5. Reduced contraction of immediate postpartum mouse myometrium by THG113. A) Tracings of spontaneous and PGF_{2α} (1 μmol/L)-induced myometrial contractility of tissues treated with THG113 or saline (control). B) Histogram depicting time-dependent change in pre- (basal) and post-PGF_{2α} (1 μM)-evoked myometrial tension in saline- and THG113-treated tissues. Values are mean ± SEM. **P* < .05 compared to corresponding value in saline-treated tissues.

duced peak, duration and frequency of myometrial contractions (Fig 4). To test the effect of THG113 on uterine contractility in the process of labor, PGF_{2α} responses on myometrial strips from CD-1 mice immediately after delivery were measured in organ baths. THG113 blocked spontaneous myometrial contractions, but only slightly reduced PGF_{2α}-induced tension; however, THG113 dissipated PGF_{2α}-induced contractions of mouse myometrium more rapidly than those observed after saline treatment (Fig 5).

Effect of THG113 on LPS-induced Premature Delivery

All saline-treated CD-1 mice delivered within 15 h after LPS injection on day 16 of gestation (term: 19 days) (Figs 6A and B). Treatment with THG113 (1 mg/day/animal) significantly delayed delivery such that 30 h later one third of animals still had not delivered, and 48 h later (corresponding to 19 days' gestation) all animals had delivered; the dose used exerted maximum efficacy as higher doses did not further delay delivery. Mouse pups from mothers treated with THG113 had increased birth weights (Fig 6C), and appeared healthy. Comparably, in mice treated with THG113 6 h after LPS injection, THG113 delayed delivery by 48 h (18.5 days gestation) in 40% of the mice (Fig 7).

A significant role for progesterone has been suggested in the onset of labor in rodents.¹⁷ PGF_{2α} is a luteolytic agent; this results in de-

creased progesterone, which in turn induces parturition in rodents.³¹ Ovariectomy of the FP-deficient mouse at 19 days' gestation induces delivery 24 h later.¹⁷ On the other hand, in humans, concentrations of progesterone that arise from placenta³¹ decrease only after placental delivery,³² and PGF_{2α} is a potent uterotonic agent.⁷ To distinguish the luteolytic and uterotonic actions of FP receptor, the effect of THG113 on mice treated with the progesterone receptor inhibitor RU486 was determined.³³ RU486 (0.1 mg/animal ip) administered on day 17 of gestation caused delivery within 12 h; on the other hand, in mice treated concomitantly with THG113 delivery was delayed until 24 h after RU486 administration (data not shown). These findings support a role for myometrial FP in parturition in rodents, consistent with other reports.^{3,7}

A number of other interactive factors in particular, cytokines, oxytocin, and other prostanoids may contribute to the process of parturition.^{31,34} Indeed, progesterone plays a key role in down-regulating FP expression,³⁵ which in turn governs the expression of oxytocin receptor¹⁷ as well as that of COX-2 in myometrium; expression of COX-2, which is FP receptor-dependent,¹⁷ results in the synthesis of PGF_{2α}, as well as of PGE₂, which exerts important uterotonic activities³⁶ largely via EP₃ receptors.^{2,37} Oxytocin seems to participate mainly in term labor, whereas PGF_{2α} exerts a predominant role

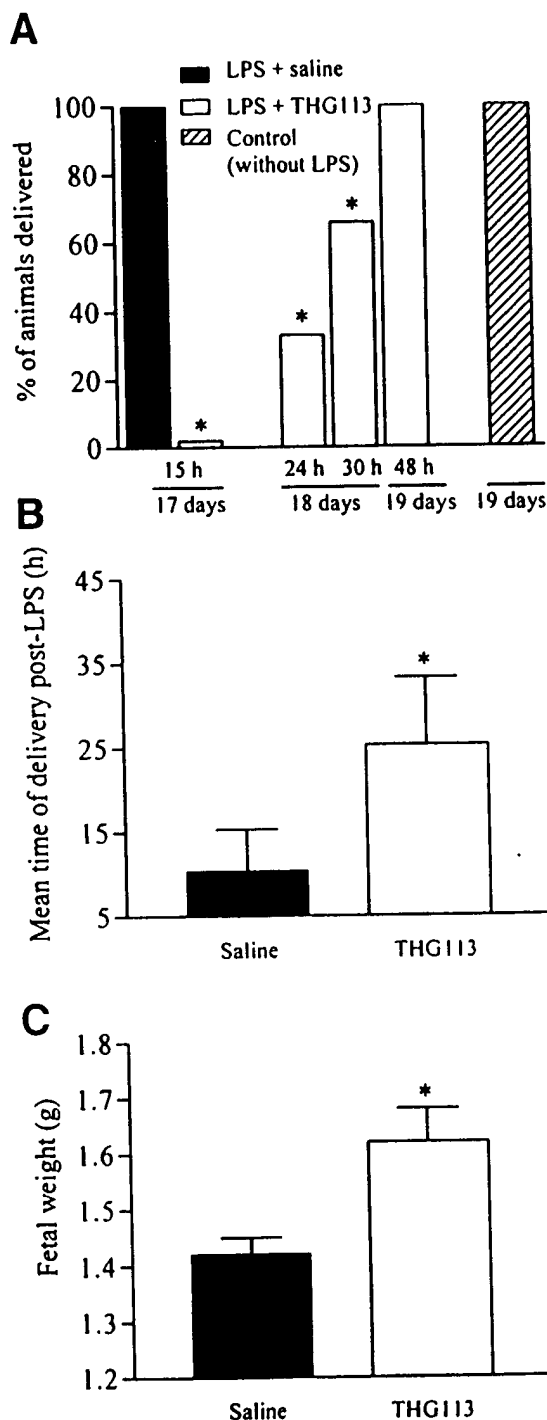


Figure 6. Tocolytic effect of THG113 in LPS-induced preterm labor in mice. Pregnant mice were injected at 16 days' gestation (term: 19 days) with LPS with or without concomitant treatment with THG113, as described in Materials and Methods. A) Percent of animals delivered; hatched bar refers to control animals not treated with LPS. Hours refer to time after LPS treatment and days to gestational age. B) Average delivery time after LPS; values are mean \pm SEM. C) Weight of pups at delivery; values are mean \pm SEM. * $P < .05$ compared to saline-treated mice.

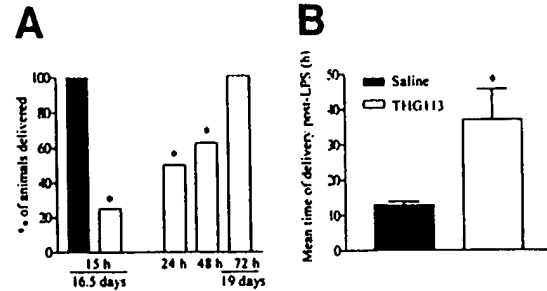


Figure 7. Tocolytic effect of THG113 4 to 7 h after administration of LPS to induce preterm labor. A) Percent of animals delivered. Hours refer to time after LPS treatment and days to gestational age. B) Average delivery time after LPS; values are mean \pm SEM. * $P < .05$ compared to saline-treated mice.

in preterm labor.⁸ The tocolytic efficacy of THG113 is consistent with the latter inference disclosing a critical role for FP in preterm parturition, particularly in preterm (present data and ref 8).

In conclusion, we have characterized THG113 as a selective, noncompetitive, reversible, and potent FP receptor antagonist. THG113 inhibited spontaneous as well as $\text{PGF}_{2\alpha}$ -induced myometrial contractions, and prolonged gestation in an endotoxin-induced preterm labor mouse model, which mimics the infectious component of preterm labor.^{28,29} Given the limited distribution of FP receptors in the organism,¹⁸ the major role of FP in parturition,¹⁷ and the relative efficacy of inhibitors of COX,^{14,16,38} including of COX-2³⁶ in tocolysis, albeit at risk of complications,^{39,40} targeting selectively FP receptor may produce efficacious and safe tocolytics for preterm labor; in this regard, THG113 is an interesting prototype.

Acknowledgment

K.G.P. and C.Q. contributed equally to the paper. The authors are thankful to Mrs Hendrika Fernandez for technical assistance.

References

1. Wright DH, Abran D, Chemtob S, et al: Prostanoid receptors: ontogeny and implications in vascular physiology. *Am J Physiol* 281:R1343-R1360, 2001
2. Crankshaw DJ, Dyal R: Effects of some naturally occur-

- ring prostanoids and some cyclooxygenase inhibitors on the contractility of the human lower uterine segment in vitro. *Can J Physiol Pharmacol* 72:870-874, 1994
3. Olson DM, Mijovic JE, Sadowsky DW: Control of human parturition. *Semin Perinatol* 19:52-63, 1995
4. Ma XH, Wu WX, Nathanielsz PW: Differential regulation of prostaglandin EP and FP receptors in pregnant sheep myometrium and endometrium during spontaneous term labor. *Biol Reprod* 61:1281-1286, 1999
5. Salmon JA, Amy JJ: Levels of prostaglandin $F_{2\alpha}$ in amniotic fluid during pregnancy and labour. *Prostaglandins* 4:523-533, 1973
6. Dray F, Frydman R: Primary prostaglandins in amniotic fluid in pregnancy and spontaneous labor. *Am J Obstet Gynecol* 126:13-19, 1976
7. Luckas M, Bricker L: Intravenous prostaglandin for induction of labour. *Cochrane Database of systematic reviews*, Issue 4, 2001
8. Cook JL, Zaragoza DB, Sung DH, et al: Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: Myometrial activation, stimulation, and preterm labor. *Endocrinology* 141:1718-1728, 2000
9. Fuchs AR, Fuchs F, Husslein P, et al: Oxytocin receptors and human parturition: A dual role for oxytocin in the initiation of labor. *Science* 215:1396-1398, 1982
10. Senior J, Marshall K, Sangha R, et al: In vitro characterization of prostanoid receptors on human myometrium at term pregnancy. *Br J Pharmacol* 108:501-506, 1993
11. Brodt-Eppley J, Myatt L: Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet Gynecol* 93:89-93, 1999
12. Miranda MA, Prince BC, Tysseling KA, et al: A proposed role for oxytocin in regulation of endometrial prostaglandin $F_{2\alpha}$ secretion during luteolysis in swine, in Ivell R, Russell J (eds): *Oxytocin*. NY, Plenum Press, 1995, pp 421-433
13. Miranda MA, Prince BC, Tysseling KA, et al: proposed role for oxytocin in regulation of endometrial prostaglandin F_2 alpha secretion during luteolysis in swine. *Adv Exp Med Biol* 395:421-433, 1995
14. Niebyl JR, Blake DA, White RD, et al: The inhibition of premature labor with indomethacin. *Am J Obstet Gynecol* 136:1014-1019, 1980
15. Morales WJ, Angel JL, O'Brien WF, et al: Use of ampicillin and corticosteroids in premature rupture of membranes: a randomized study. *Obstet Gynecol* 73:721-726, 1989
16. Carlan SJ, O'Brien WF, O'Leary TD, et al: Randomized comparative trial of indomethacin and sulindac for the treatment of refractory preterm labor. *Obstet Gynecol* 79:223-228, 1992
17. Sugimoto Y, Yamasaki A, Segi E, et al: Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277:681-683, 1997
18. Coleman RA, Smith WL, Narumiya S: International union of pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46:205-229, 1994
19. Abramovitz M, Adam M, Boic Y, et al: The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim Biophys Acta* 1483:285-293, 2000
20. Chemtob S, Peri K: THG113. *BioContact Meeting*, Québec, 2000
21. Bhattacharya M, Peri KG, Chemtob S, et al: Nuclear localization of prostaglandin E_2 receptors. *Proc Natl Acad Sci USA* 95:15792-15797, 1998
22. Abran D, Varma DR, Chemtob S, et al: Reduced responses of retinal vessels of the newborn pig to prostaglandins but not to thromboxane. *Can J Physiol Pharmacol* 72:168-173, 1994
23. Abran D, Varma DR, Chemtob S: Increased thromboxane-mediated contractions of retinal vessels of newborn pigs to peroxides. *Am J Physiol* 268:H628-H632, 1995
24. Abran D, Varma DR, Chemtob S: Regulation of prostanoid vasomotor effects and receptors in choroidal vessels of newborn pigs. *Am J Physiol* 272:R995-R1001, 1997
25. Chemtob S, Aranda JV, Varma DR: Effects of prostaglandins on isolated internal carotid and basilar arteries from newborn and adult pigs. *Pediatr Res Commun* 4:101-109, 1989
26. Chemtob S, Inayatulla A, Varma DR: Eicosanoid-dependent and endothelium-independent oscillations of rat aorta. *J Vasc Res* 29:270-280, 1992
27. Varma DR, Chemtob S: Endothelium- and beta-2 adrenoceptor-independent relaxation of rat aorta by tyramine and certain other phenylethylamines. *J Pharmacol Exp Ther* 265:1096-1104, 1993
28. Romero R, Roslansky P, Oyarzun E, et al: Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. *Am J Obstet Gynecol* 158:1044-1049, 1988
29. Romero R, Sirtori M, Oyarzun E, et al: Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. *Am J Obstet Gynecol* 161:817-824, 1989
30. Narumiya S, Sugimoto Y, Ushikubi F, et al: Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 79:1193-1226, 1999
31. Soloff MS: Cellular and integrative mechanisms, in McNellis D, Challis J, MacDonald P, Nathanielsz P, Roberts J (eds): *The onset of labor*. Ithaca NY, Perinatology Press, 1988, pp 87-123
32. Tulchinsky D, Hobel CJ, Yeager E, et al: Plasma estradiol, estriol, and progesterone in human pregnancy. II. Clinical applications in Rh-immunization disease. *Am J Obstet Gynecol* 113:766-770, 1972
33. Fang X, Wong S, Mitchell BF: Effects of RU486 on estrogen, progesterone, oxytocin, and their receptors in the rat uterus during late gestation. *Endocrinology* 138:2763-2768, 1997
34. Kimura T, Ogita K, Kusui C: What knockout mice can tell us about parturition. *Rev Reprod* 4:73-80, 1999
35. Ou CW, Chen ZQ, Qi S: Expression and regulation of the messenger ribonucleic acid encoding the prostaglandin $F(2\alpha)$ receptor in the rat myometrium during pregnancy and labor. *Am J Obstet Gynecol* 182:919-925, 2000
36. Gross G, Imamura T, Vogt SK, et al: Inhibition of cyclo-

- oxygenase-2 prevents inflammation-mediated preterm labor in the mouse. *Am J Physiol* 278:R1453-R1459, 2000
37. Smith GS, Baguma-Nibasheka M, Wu WX, et al: Regional variations in contractile responses to prostaglandins and prostanoid receptor messenger ribonucleic acid in pregnant baboon uterus. *Am J Obstet Gyn* 179:1545-1552, 1998
38. Morales WJ, Smith SG, Angel JL, et al: Efficacy and safety of indomethacin versus ritodrine in the management of preterm labor: A randomized study. *Obstet Gynecol* 74: 567-572, 1989
39. Takahashi Y, Roman C, Chemtob S, et al: Cyclooxygenase-2 inhibitors constrict the fetal lamb ductus arteriosus both in vitro and in vivo. *Am J Physiol* 278:R1496-R1505, 2000
40. Norton ME, Merrill J, Cooper BA, et al: Neonatal complications after the administration of indomethacin for preterm labor. *N Engl J Med* 329:1602-1607, 1993

SEMINARS IN PERINATOLOGY

VOL 26, NO 6

DECEMBER 2002

INTRODUCTION

THE INTERNATIONAL Perinatal Collegium is a body of colleagues from many parts of the world that meets every 2 years to exchange new research findings in the areas of perinatology and neonatology. The organizers of this group are Dr Bill Oh from Providence, RI, Dr David Schiff from Edmonton, Alberta (Canada), and Dr Paul Vert from Nancy (France). The meeting is made possible by the generous support of Mead Johnson Nutritionals. The most recent meeting of this Collegium was held on Marco Island, FL, from July 21-25, 2001, when 50 members and guests from 15 countries came together for a lively and provocative scientific exchange. Eleven papers presented at the meeting were selected for inclusion in this issue of *Seminars in Perinatology*.

The first 2 papers in this issue focus on eicosanoid biology. The cyclooxygenase (COX) inhibitor indomethacin is commonly used as a tocolytic, but its efficacy is limited and its use is associated with significant adverse effects in the fetus and newborn. Dr K.G. Peri and colleagues have taken a different approach to the prevention of premature labor with their investigations of a receptor antagonist for one of the COX products, PGF_{2α}, which is known to play a pivotal role in parturition. In their article, they describe the pharmacological properties of this receptor antagonist, THG113, and show its ability to delay preterm delivery in a mouse model of endotoxin-induced premature labor.

Indomethacin is also used to prevent or treat symptomatic patent ductus arteriosus in newborn premature infants. The use of indomethacin in this context is associated with significant adverse effects, particularly decreased renal function. Another COX inhibitor, ibuprofen, has been recommended as an alternative to indomethacin because several clinical trials have

shown less effect on renal function with ibuprofen than with indomethacin. However, Dr Jean-Pierre Guignard showed in 6-day-old rabbits that ibuprofen had adverse effects on renal function that were no different from those occurring with indomethacin and aspirin. These results emphasize that neonatologists should be concerned about adverse effects on renal function regardless whether indomethacin or ibuprofen is chosen for use in the management of symptomatic patent ductus arteriosus.

The question whether we are doing more harm than good when we administer 100% oxygen to asphyxiated infants in the delivery room has received further examination by Dr Máximo Vento and colleagues. They report evidence of increased oxidative stress in asphyxiated infants resuscitated with 100% oxygen. These findings raise the possibility that hypoxic ischemic encephalopathy, which involves reperfusion-generated free radical injury, might be further aggravated by resuscitation with high concentrations of inspired oxygen. Neonatologists may be exposing infants inadvertently to the adverse effects of oxygen by means of another common neonatal intensive care unit (NICU) intervention—blood transfusion. In their article, Dr Virginie De Halleux and colleagues show that the decreased oxygen affinity after transfusion with adult red blood cells increases the amount of oxygen available to the immature tissues of preterm infants. They go on to offer the provocative recommendation that lowering oxygen saturation targets after a transfusion may protect against hyperoxygenation and free radical damage.

Another toxin to the immature neonate is

Copyright 2002, Elsevier Science (USA). All rights reserved.
doi:10.1053/sper.2002.37142

bilirubin. By using cultured neurons from the developing rat brain, Dr Stéphanie Grojean and colleagues show the adverse interaction of free bilirubin and hypoxia on cell viability and apoptosis, protein synthesis, and energy metabolism. Not only did the combination of bilirubin with hypoxia result in stronger detrimental effects on neurons than bilirubin or hypoxia alone, bilirubin was also shown to prevent the adaptive cell response to sublethal hypoxia. In view of these findings, would Dr De Halleux group's recommendation for lower oxygen saturation targets also apply after an exchange transfusion for hyperbilirubinemia?

Mechanical ventilators now in use in NICUs often display a variety of electronically derived information such as real-time flow-volume and pressure-volume graphs. Dr Hugo Devlieger and colleagues have added a third graph—flow v pressure—to the ventilatory information available at the bedside of infants being mechanically ventilated. In their article, they describe how the flow-pressure graph can be used to detect subtle changes in infant-ventilator interaction. This information could lead to further refinements in the bedside management of mechanical ventilation and help reduce some of the lung injury inflicted by mechanical ventilation.

Most neonatologists think of nitric oxide as a molecule involved in the modulation of pulmonary vascular tone. In their article, Dr Richard J. Martin and colleagues call our attention to the effects of NO on airway smooth muscle tone. Of particular interest is that the ability of endogenously released NO to oppose airway constriction may be impaired in response to hyperoxia. This and other projected benefits of inhaled NO on airway function may become recognized as a result of clinical trials currently underway evaluating the efficacy of inhaled NO in the prevention and treatment of BPD.

Achieving adequate nutrition in premature infants, especially those with compromised gastrointestinal function, remains a significant challenge for neonatologists. Often, partially hydrolyzed formulas are used to facilitate intestinal absorption of nutrients. However, these formulas result in decreased nitrogen and calcium absorption rates compared with standard premature formulas. To address this problem, Dr Jean-

Charles Picaud and colleagues evaluated a new formula with modified nitrogen and calcium sources. In their article, they report that an appropriate nitrogen retention and plasma amino acid profile can be achieved with the new formula.

It has been suspected for decades that the risk of respiratory distress syndrome (RDS) may be affected by genetic factors. Two papers in this issue address this question. In their article, Dr Loekie van Sonderen and colleagues present data from a twin study supporting this suspicion. They showed that RDS occurred more frequently in both twins when the twins were monozygotic than when the twins were dizygotic. In the other article, Dr Mikko Hallman and colleagues were unable to show any major direct genetic influence when they screened nearly one million birth records of Finnish origin to identify premature multiple births with at least one RDS infant. Their findings appear to contradict the van Sonderen report and the growing body of evidence that certain genetic polymorphisms in surfactant proteins A and B increase RDS risk. However, they go on to argue that this apparent conflict can be resolved by taking into consideration birth order of twins and differences in environmental factors such as cytokine exposure between the first and second born twin.

In the final article, Dr Bobbi J. Byrne and colleagues present epidemiological evidence of a decline in the risk of bronchopulmonary dysplasia in their NICU since the beginning of 1991. Prior to 1991, improving survival of very low birth weight infants was accompanied by a proportional increase in the incidence of BPD. The reversal of this trend in the past decade was associated with a variety of changes in clinical practice. If these findings are representative of other NICUs, they signify an important reduction in the impact of BPD as one of the costly sequelae of prematurity.

We hope that this selection of papers from the 2001 International Perinatal Collegium will be of interest to the readership of *Seminars in Perinatology*.

ROBERT B. COTTON, MD
Guest Editor